Jl S. Afr. Bot. 37 (3): 157–168 (1971)

DEVELOPMENTAL MORPHOLOGY OF A NEW LOCULOASCOMYCETE ON *EUPHORBIA.*

Stephen K-M. Tim

(*Department of Botany & Microbiology, Rhodes University, Grahamstown*)

ABSTRACT

A new genus in the Loculoascomycetes is described. Infection is initiated by germ tubes from ascospores penetrating the stomata on the stems of the host. Stromata eventually appear above the stomata and from these, plates of prosenchyma form parallel with the stem surface. More stromata arise from these plates of cells. Centrum development of the ascocarp is of the Dothidea type, with the asci growing up into a space which results from the disintegration of pseudoparenchyma. An unusual feature in the development of the various structures is the prevalence of intracellular cleavage of multinucleate cells to form uninucleate ones.

UITTREKSEL

DIE ONTWIKKELINGS MORFOLOGIE VAN 'N NUWE LOCULOASCOMYCETE OP *EUPHORBIA.*

'n Nuwe genus in die Loculoascomycetes word beskrywe. Besmetting word deur kiembuise van askospore, wat die stomatas op die stamme van die gasheer, binne dring aan die gang gesit. Stromas verskyn uiteindelik bo die stomatas en hieruit, ontwikkel plate van prosenchiem wat parallel met die oppervlak van die stam groei. Verdere stromas ontwikkel dan uit hierdie plate van selle. Die ontwikkeling van die sentrum van die askokarp geskied volgens die Dothidea tipe: die askospore groei in 'n ruimte wat as gevolg van die afbreek van die dieper weefsel ontstaan. 'n Buitengewone kenmerk in die ontwikkeling van die verskillende strukture is die frekwente intrasellulêre verdeling van veelkernige selle om eenkernige selle te vorm.

INTRODUCTION

This fungus described here is parasitic on the stems of *Euphorbia bothae* Lotsy & Godd. Uniloculate ascostromata, interspersed with microconidial locules, form superficially, in concentric rings, upon the epidermis (figs. 1A, B).

Communication with the National Herbarium, Pretoria, (Marasas, 1969—Personal communication) has revealed that the only Ascomycete recorded on *E. bothae* is one with a pulvinate stroma with ascigerous locules arranged in a peripheral layer on this stroma and has been labelled as '*Dothidea* sp. indet'. These stromal characteristics do not compare with this fungus being studied.

In the keys of Clements and Shear (1957), this fungus cannot be accommodated easily into any of the genera listed in the phaeodidymous Sphaeriaceae or Dothideaceae. Employing the keys of Müller and von Arx (1962) this fungus is best placed in the Pseudosphaeriales and in the Dimeriaceae or possibly,

Accepted for publication 19th January, 1970.

the Mycosphaerellaceae. However, in the genera in both families closest to this fungus in description, these are invariably featured as having 'paraphysoids' among the asci. As interascicular structures are lacking in this fungus, it cannot be accommodated in either family.

Since, after an examination of modern literature, this species could not be placed in a suitable genus, it was decided to describe it as a new genus and species.

SCUTELLOIDEA TIM, gen. nov., (Dothideaceae, sensu Luttrell, 1955).

Stromata superficialia, unilocularia, atrobrunnea sive nigra. Stromata acervis solutaris cellarum coperta. Loculi in stromatibus formati quae et in et super stomatibus crescent vel gignunt ex incremento laminarum prosenchymatis quae ipsae ex stromatibus evolvunt et paralleles superficiei hospitis oriuntur. Asci cylindrici, octospori, bitunicati, aparaphysati. Sporae brunneae, bicellulares.

Type species: *S. concentrica* Tim.

Stromata superficial, uniloculate, brown to black in colour. Stromata covered in detachable clumps of cells. Locules formed in the stromata which in turn arise in and above stomata or as a result of the proliferation of the plates of prosenchyma which arise from the stromata and grow parallel with the surface of the host. Asci cylindrical, eight spored, bitunicate, aparaphysate. Ascospores brown, bicellular.

Etymology:	L.	scutellum=shield.
	Gk.	eidos=form.

Scutelloidea concentrica Tim, sp. nov.

Stromata caulicola, superficialia, uniloculata, saepe confluentia, 88—113=110—175μ, ex medio evoluta et plus minusve concentrice disposita. Asci octospori, cylindrici, 75—88×20—25μ, bitunicati, aparaphysati. Sporae medio vel paullo supra medium septatae, leniter constrictae, 25—30×6—8μ.

Type: South Africa, Cape—3326A (Grahamstown): Hell's Poort, *Tim* 198 (PRE, holo.; K, iso.).

Stromata caulicolous, superficial, uniloculate, may be confluent, 88—113×110—175μ, developing centrifugally and arranged in concentric rings. Asci eight spored, cylindrical, 75—88×20—25μ, bitunicate, aparaphysate. Spores two celled, medially septate or cells subequal, very slightly constricted, 25—30×6—8μ.

Etymology:	L. cum=together; centrum=centre.
Habitat:	Stems of *Euphorbia bothae* Lotsy and Godd.

MATERIALS AND METHODS

Infected stems of *Euphorbia bothae* were collected on a farm in Hell's Poort, Grahamstown during the period July to January, 1965—1966, 1966—1967. The earliest stages in development were usually evident during August whilst the mature stages were present throughout the year.

Slices of tissue containing stromata $\frac{1}{8}''$ (or 3 mm) in thickness, were removed and the excess latex wiped away. It was important that the latex did not cover the stromata as this would have trapped particles of grit among the fruiting bodies which would interfere with the process of sectioning. Small squares of infected tissue were fixed in form-acetic-alcohol, carried through a butyl alcohol series and imbedded in 55—60° wax. Sections were cut at 6—10μ and stained in Heidenhain's haematoxylin with a counterstain of Orange G (Johansen, 1940). Temporary preparations were mounted in cotton blue in lactophenol.

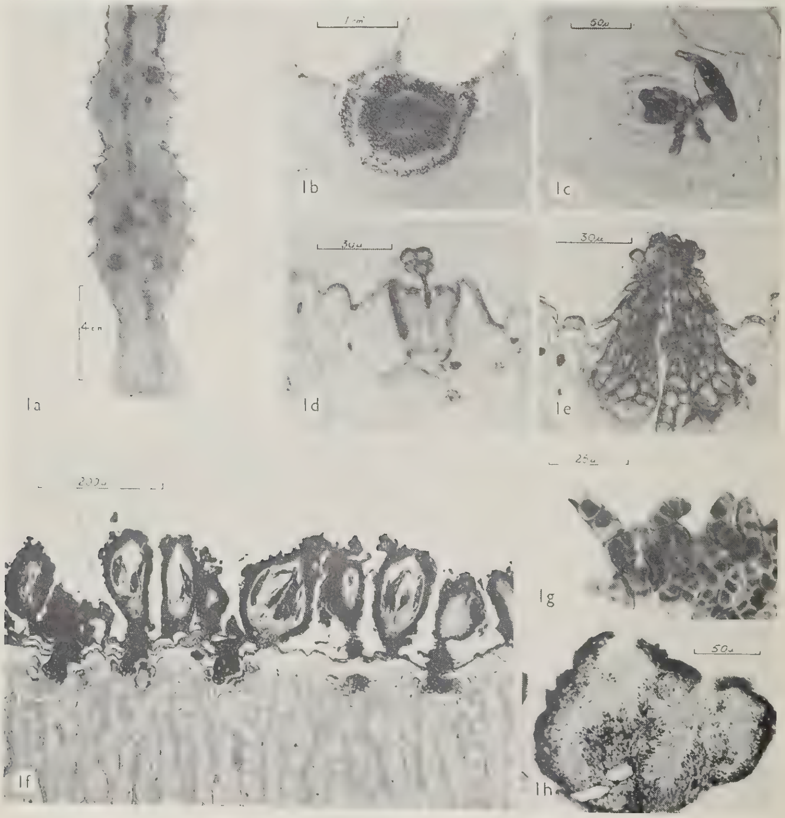
For the examination of the details of spore germination on the stem, infected areas were coated with two layers of clear nail varnish and left to dry for 12 hours. The strips were carefully removed after freeing the edges with a scalpel and mounted in cotton blue in lactophenol.

Host-Parasite Relations

Ascospores are produced in abundance from the numerous ascocarps which cover large areas of the stem. However, very few of these spores actually germinate on the stem, and of those which do, only a small proportion gain entrance into the host. To elucidate some of the factors governing spore germination, the following was carried out: Segments of infected stem were positioned about 5 cm above clean stems selected from the tops of the plants. These were left overnight in a moist atmosphere to induce spore ejection onto the uninfected stems. After this treatment, the stems were placed outdoors in a protected position and at three day intervals, a nail varnish strip was made of the newly infected part.

The number of spores germinating with time did not increase significantly. Spores which germinated near stomata exhibited a more precocious development than those which germinated away from stomata. The same was found for spores germinating near a crack in the cuticle. It was thus concluded that the unusually thick cuticle afforded an effective barrier against invasion by this fungus. The fact that germ tubes ramified between cracks in the cuticle seemed to indicate that it was unlikely that invasion through stomata was due to stimulation by a specific factor or set of factors associated with stomata as spore germination occurred near to as well as away from stomata. When germ tubes were produced some distance from a stoma, they showed randomised growth and were light in colour. Those tubes near a stoma were darker in colour, penetrated into the opening directly and produced a small knot of dark cells above the guard cell (figs. 1C, D). From such a knot, a hypha would grow inward between the guard cells (fig. 1D) and invade the substomatal cavity, filling this with hyphae. Invasion of the host takes place from this part and hyphae penetrate between the cells, entering the deeper tissues of the host. At all times, the hyphae, which are composed of multinucleate cells, remain intercellular, do not have haustoria and do not penetrate very deeply into the tissue; the infected area remains relatively superficial and there is no attendant hypertrophy evident.

Subsequent to the hyphal invasion of the internal tissues, dark aggregations of hyphal cells concentrate near a number of stomata in close proximity to



each other. These fungal cells increase and displace the guard cells in the process (fig. 1E) and are the initials of the fruiting bodies. In other words, the fungus gains access into the host by way of the only natural openings, the stomata. After invasion of the tissue, these stomata serve as the exits for the fungus and it is on the surface of the stem that further development takes place (fig. 1F).

Each stromal initial proliferates, forming a cone of pseudoparenchyma (fig. 1E). From this cone, the outermost cells in localised areas divide, forming paradermal plates of tissue mostly one cell in thickness and these lie in parallel sheets (figs. 1E, F) raised a short distance from the surface of the stem. The appearance of a young stroma in vertical section is very much that of a pagoda.

Proliferation of the hyphae of the plates occurs by means of intracellular cleavage. In each cell, the protoplasm is seen to divide into a number of separate portions (fig. 1G). Each portion may again divide so resulting in a bulging-out of the wall of the parent cell. In this way, the prosenchyma becomes propagated in all directions. The plates retain their hyphal construction for most part and coalesce with adjacent plates. At any point between stomata, the cells of these plates may proliferate and form a knot of pseudoparenchyma which also gives rise to a fruiting body and hence the number of fruiting bodies per unit area becomes considerable (fig. 1F). In the formation of a stroma, a few of the hyphae of the plates grow upward and undergo a series of intracellular cleavages to form a knot of cells. The process continues, with more cells being added to the developing stroma. Even when the fruiting structures, whether ascocarps or

---

Fig. 1A.

Infected portion of the stem of *Euphorbia bothae*, with compact masses of uniloculate ascostromata and microconidial locules grouped on the stem surface.

Fig. 1B.

A single infection showing the concentric arrangement of bands of fruiting bodies.

Fig. 1C.

Germinated ascospore near a stoma. A knot of hyphae is produced from the germ tube just above the stoma.

Fig. 1D.

Hypha growing out from the knot of hyphae above a stoma and penetrating between the guard cells of the host.

Fig. 1E.

Cone of pseudoparenchyma of a stromal initial formed in the immediate region of a stoma and displacing the guard cells. Paradermal plates of tissue are produced from the stromal mass.

Fig. 1F.

Vertical section through an infection showing the superficial, uniloculate ascostromata formed from the stromal proliferations through stomata or from stromata formed from the paradermal plates.

Fig. 1G.

Cleaved protoplasts of cells of the paradermal plates.

Fig. 1H.

Vertical section through a multiloculate microconidial locule. Microconidia escape throu a number of openings.

microconidial locules, are mature the surface cells continue this protoplasmic cleavage (fig. 2A) so resulting in the surface being covered with these muriform aggregations of cells. However, they differ from the cells forming the parallel plates in that these cells which have undergone protoplasmic cleavage do not go on dividing indefinitely to form plates of tissue but remain as loose clumps of cells which add to the bulk of the fruiting bodies and are easily brushed off from the surface. Their role, if any, in the biology of the fungus is unknown.

The fruiting bodies, to begin with, form a dense patch, occupying a circular area up to 3 mm in diameter. Eventually, the infection spreads, still in a circular manner, with a relatively clear zone remaining between the original infection and the next band. This process may continue till the area of fruiting bodies is some 2 cm in diameter, consisting of from four to six separate bands (fig. 1B). The concentric banding itself may be due to seasonal effects. From previous observations, only ascospores germinating near stomata produce an infection. The chance of a number of stomata in close proximity being infected is highly likely even under natural conditions, as the volume of spores produced from such abundant stromata must, logically, be tremendous. What remains puzzling is why a considerable area of stem is not covered by fruiting bodies from the start and why coalescent circles of infection are relatively rare. Old parts of the stems do show a considerable intergrading of circles of the fungus but this is the result of the original rings increasing in number with time. Perhaps there is some sort of inhibitory reaction whereby the first spore to gain access into the plant sets up a barrier which inhibits the further development of other germinated spores in the immediate vicinity.

Microconidial locules

The first fruiting bodies to be produced during the early phases of infection are the microconidial locules. In the cone-shaped mass of pseudoparenchyma formed above each stoma, a lighter staining portion in the centre becomes obvious. On closer examination when the structure is of a reasonable size, fertile portions producing microconidia can be seen to arise in a number of parts within the central, lighter staining part. As each of the parts producing microconidia develops at a different rate, the structure appears to be multiloculate. Because of its amorphous shape (fig. 1H) a microconidial locule is clearly distinguishable from an ascigerous locule and at maturity measures $50—110\times65—135\mu$. The same procedure in locule formation takes place in the stromata developed on the hyphal plates between stomata.

The formation of microconidia takes place centrifugally. Microtome sections did not provide conclusive information as to the origin of the microconidial mother cells; squash preparations of young stromata proved far more satisfactory here.

Initially, in each locule, the pseudoparenchyma takes on a loose appearance and each cell can be seen to have dense protoplasm with a number of nuclei. Each cell destined to form microconidial mother cells becomes somewhat elongated and points toward the centre of the locule. The protoplast undergoes cleavate so forming a row of segments (fig. 3A). Cell walls eventually form and the whole structure consists of a row of cells, each with a single nucleus. The squash preparations showed most of the developmental stages. Individual portions in each of the cleaved cells stained up with the cotton blue whilst the wall of the original cell investing the portions remained hyaline.

After wall formation, each portion becomes a mother cell. Microconidia are first produced from the terminal cell. The tip of this cell becomes drawn out to form a symmetrically placed sterigma from which a bacilliform microconidium is cut off. The microconidia are uninucleate, 1,8—2,5 × 0,7—0,9μ, and even when still attached to the mother cell, the latter is seen to contain a prominent nucleus. It can only be presumed that each mother cell is capable of giving rise to more than one microconidium. Cells lower down the series may also produce sterigmata. It seems logical to conclude that after a number of microconidia have been formed, each mother cell disintegrates, its role being continued by other cells further down the series. Cells deeper in the pseudoparenchyma lining the locule assume similar functions after their protoplasts cleave. This would account for the progressive enlargement of the locule with the concomitant growth in size of the stroma.

The microconidia fill the locule and ooze out through a number of irregular openings which break through the outer layers of the stroma. The actual role of these microconidia in this fungus is presumed to be spermatial. Their small size and abundant production are features similar to structures in other fungi where their spermatial function has been ascertained.

Ascigerous Locules

Ascostromata are initiated in the same way as the microconidial locules. Mounds of pseudoparenchyma arise either above the stomata or form as a result of the proliferation of the prosenchyma lining the surface of the host. Whilst stromata destined to become microconidial locules show the presence of microconidia at a very early age in the lighter staining central mass of cells, ascostromal masses appear more compact to begin with. The central mass soon loses its compactness and the cells appear almost separated from one another (fig. 2A). Among these cells, a loose, partially coiled, multinucleate structure arises with a projection reaching up toward the apex. In a few cases, these projections appeared to penetrate the outer, dark layers of the stroma and to reach the outside (fig. 3B). This structure is possibly the ascogonium with trichogyne.

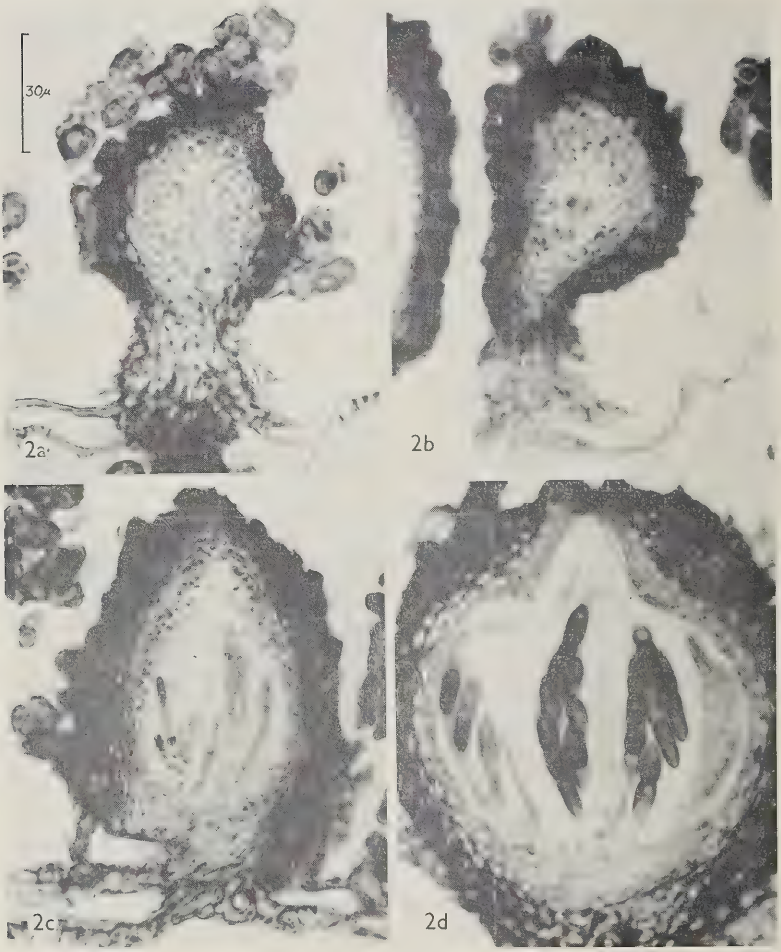


Fig. 2A.

Vertical section through a young ascostroma showing a separation of the internal pseudoparenchyma cells. Note the masses of cleaved protoplasts, still invested by the original cell walls, clustered around the outside of the fruiting body.

Fig. 2B.

Lobed ascogonium, with a number of pairs of nuclei, situated in a space which has resulted from the distintegration of the centrum pseudoparenchyma. Note the inwardly pointing rows of cells originating from the pseudoparenchyma lining the dark outer layers of the ascostroma.

Fig. 2C.

Branched ascogenous system from which asci arise. Each ascus has a fusion nucleus.

Fig. 2D.

Mature ascostroma with prominent ostiolar area at the top.

The area around the ascogonium becomes progressively more open and soon, is surrounded by a space with the cells lining the inner part of the ascocarp pointing inwards (fig. 2B). These latter cells are elongate and consist of a number of fragmented portions of the protoplast of single cells with each fragment having its own nucleus. Cell walls eventually delimit these portions in a linear series in the same way as was noted for the microconidial mother cells. Due to their limited growth these chains of cells cannot be considered homologous with pseudoparaphyses (sensu Luttrell, 1965). The formation of the space around the ascogonium comes about as a result of the breakdown of the centrum cells. Remains of these cells in the form of extremely fine cell walls, devoid of contents, can be detected around the ascogonium. These often appear as fine threads connecting the ascogonium to the surrounding stromal layers.

Figure 2B shows a lobed ascogonium with a number of pairs of nuclei, indicating that plasmogamy has already occurred. Cell walls are not apparent and the ascogonium is still multinucleate. Cell walls eventually form and the ascogonial complex is then made up of a number of cells in a chain-like series, somewhat reminiscent of the cells from which the microconidia arise. Short lateral branches grow out from some of the individual cells and the binucleate condition becomes perpetuated into these arms. These constitute the ascogenous hyphae and radiate out from a complex of cells toward the base of the locule. Such a complex of ascogenous cell from which asci arise, was also recorded in *Dothidea collecta* (Luttrell, 1951a).

In each of these short ascogenous hyphae, the two nuclei fuse directly, giving rise to the fusion nucleus in the ascus initial. Croziers are not formed. This ascus initial becomes much enlarged and vacuolate and the single fusion nucleus stands out prominently (fig. 2C). As ascus development progresses, the whole locule enlarges and the clear area around the ascogenous system becomes wider allowing for the ingrowth of asci to carry on without hindrance from extraneous tissue. At the apex, a beak develops and is lined with rows of cells similar to those which line the rest of the locule. These, however, are longer and persistent and remain as the periphyses whereas the others in the main body of the locule soon lose their identity, possibly as a result of the crowding by asci (fig. 2D). A pore finally breaks through the dark layers of the ascocarp. The latter, at maturity, is perithecium-like in form and of dimensions 88—113 × 110—175μ. Eight bicellular ascospores are produced in each ascus. The spores are dark in colour, 25—30 × 6—8μ, and are composed of two equal to subequal cells with a waist at the septum. The asci are cylindrical (fig. 3C), 75—88 × 20—25μ, and bitunicate (fig. 3D) and on extension, the endoascus of each ascus in turn reaches through the pore and the spores are shot out in quick succession.

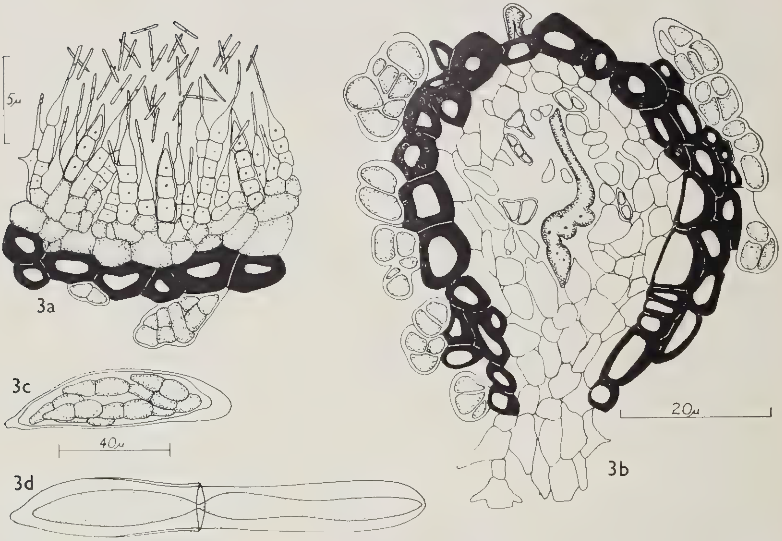


Fig. 3A.
Microconidial mother cells originating as cleaved portions of the protoplasts of cells lining a locule. A sterigma, from which microconidia are produced, is usually formed from each of the portions.

Fig. 3B.
Ascogonium with trichogyne in a young ascostroma.

Fig. 3C.
Unextended ascus with ascospores.

Fig. 3D.
Ascus after extension of endoascal wall through the ectoascal wall. Spores already ejected.

Discussion

The early ascigerous locule consists of a compact mass of pseudoparenchyma composed of thick walled peripheral cells devoid of stainable contents and thin-walled, nucleated cells of the centrum. Within the latter, an ascogonium becomes differentiated and such development would make the fruiting structure an ascostroma. This, together with the presence of the bitunicate ascus would place *Scutelloidea concentrica* in the Loculoascomycetes (Luttrell, 1955).

The fact that this fungus is described in a new genus, the specific definition of the general stroma is important. The initial stromata emerge from the stomatal openings and are positioned superficially on the surface of the host. From these stromata, plate of prosenchyma grow out, invest the surface of the stem and further stromata form as a result of the proliferation of cells of these plates. These parallel plates would therefore also constitute the stroma. The uniloculate ascostromata would be correctly described as being situated above stomata or on a stromal base arising from plates of prosenchyma which lie parallel with the stem of the host.

Disruption of the centremost cells of the centrum progresses to the point where the asci grow up into a space. Interascicular structures such as paraphyses, pseudoparaphyses, interthecial tissue (sensu Luttrell, 1965) are absent. Such development would conform with the Dothidea type and would place *Scutelloidea* in the Dothideales (Luttrell, 1951b). It would also be conveniently included in the Dothideaceae due to the perithecium—like locule and fasciculate arrangement of asci.

The frequent occurrence of protoplasmic cleavage as a form of cell division in *Scutelloidea concentrica* is a feature shared with *Dothidea collecta* (Luttrell, 1951a). It appears that almost all the cells, to begin with, are multinucleate. As a result of intracellular divisions, the uninucleate condition is brought about in all cells with the exception of the ascogenous hyphae where binucleate cells are delimited.

ACKNOWLEDGMENTS

This paper is based on a dissertation submitted to Rhodes University in partial fulfilment for the degree of Doctor of Philosophy. This work was supported in part by grants from the C.S.I.R. and Rhodes University.

Grateful thanks to Professor E. S. Twyman for his supervision throughout the course of this work. Thanks are also due to Mr. J. P. Jessop for his helpful criticism and to Dr. B. J. Cholnoky and Prof. B. Dietrich for their checking of the Latin diagnosis.

REFERENCES

CLEMENTS, F. E. and SHEAR, C. L., 1931. *The Genera of Fungi.* New York: Hafner Publishing Co.

JOHANSEN, D. A., 1940. *Plant Microtechnique.* New York: McGraw-Hill.

LUTTRELL, E. S., 1951a. The morphology of *Dothidea collecta. Amer. J. Bot.* **38:** 460–471.

————, 1951b. Taxonomy of the Pyrenomycetes. *Univ. Mo. Stud.* **24** (3): 1–120.

————, 1955. The Ascostromatic Ascomycetes. *Mycologia* **47:** 511–532.

————, 1965. Paraphysoids, pseudoparaphyses, and apical paraphyses. *Trans. Br. mycol. Soc.* **48:** 135–144.

MÜLLER, E. and VON ARX, J. A., 1962. Die Gattungen der didymosporen Pyrenomyceten. *Beit. Kryptogamenflora Schweiz II* **2:** 1–885.